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Acanthophora dendroides Harvey (Rhodomelaceae), a new record for the Atlantic and Pacific oceans

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Abstract

We record *Acanthophora dendroides* Harvey for the first time in the Atlantic Ocean. Two specimens from the Philippines were resolved as conspecific to the Atlantic *A. dendroides* in molecular analyses extending its geographic range to the Philippines. In light of new evidence provided by field-collected specimens of *Acanthophora spicifera* (M.Vahl) Børgesen (generitype) from Florida and Venezuela, the flattened species *A. pacifica* (Setchell) Kraft, showed no affinity to *Acanthophora* sensu stricto, suggesting that the genus should be restricted to cylindrical species only.

Key words

Atlantic Ocean, Philippines, taxonomy.

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Introduction

The genus *Acanthophora* J.V. Lamouroux 1813 is a member of the tribe Chondrieae and it is distinguished from other genera of the tribe by the presence of spirally arranged acute spines (Gordon-Mills and Womersley 1987). The genus is widely distributed in tropical regions with 7 currently accepted species (de Jong et al. 1999). Species with an apparent cosmopolitan distribution such as *Acanthophora spicifera* (M. Vahl) Borgensen (the generitype) and *A. muscoides* (Linnaeus) Bory appear to be common components of the marine flora in many coastal areas around the world (Guiry and Guiry 2018). *Acanthophora nayadiformis* (Delile) Papenfuss is a common species in the Mediterranean Sea and some coastal areas of East Africa (Cecere and Perrone 2002). In contrast, other species are geographically restricted. *Acanthophora aokii* Okamura and *A. pacifica* (Setchell) Kraft are restricted to the Pacific Ocean (Guiry and Guiry 2018), *A. dendroides* Harvey to the Indian Ocean (Silva et al. 1996) and *A. ramulosa* Lindenb. ex. Kutzing appears to be confined to the Gulf of Guinea in West Africa (Steentoft 1967).

Affinities among *Acanthophora* species have been based on morphological characters using cladistic analysis (de Jong et al. 1999). Morphological characters such as spine development and tetrasporangial branches features have been historically used for species delimitation (Harvey 1855, Falkenberg 1901, Steentoft 1967, de Jong et al. 1999). However, Cecere and Perrone (2002) and Perrone et al. (2006) concluded that these features have no diagnostic value and the gross morphology of thalli in the same species is deeply affected by the type of growth pattern (whether monopodial or sympodial) and primordium type.

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Acanthophora dendroides was originally described by W.H. Harvey in 1855 from Western Australia (Indian Ocean). Currently, this species has a disjunct distribution in the Indian Ocean and it is known from East Africa, South West Asia, and Australia (Guiry and Guiry 2018). We report a fourth species of *Acanthophora*, *A. dendroides,* for the East coast of Florida based on molecular and morphological evidence. Likewise, we extend the geographic range of this species to the Philippines, where it has not been previously recorded, accounting for the first report of the species in the Pacific Ocean.

Methods

Collection and vouchers. Specimens were collected along the intertidal zone from localities in Venezuela and Florida, preserved in 4% formalin seawater for anatomical studies and portions were desiccated in silica gel for DNA extraction. Collection, voucher information, and GenBank accession numbers of newly collected material and Philippine specimens are provided in Table 1.

Anatomical studies. Anatomical observations were made on liquid preserved material and herbarium sheets. Wet mounts of apical portions and handmade cross sections were prepared unstained or stained with a 1% acidified (HCl) aniline blue solution. Preparations were examined under the light microscope. Photographs were taken in a stereoscope and light microscope with an integrated camera Olympus Q-color 3 (Olympus America, Center Valley, PA), edited and assembled into plates using Adobe Photoshop CC (Adobe Systems Incorporated).

DNA extraction, gene amplification and sequencing. Genomic DNA was extracted from samples preserved in silica gel using the Cetyl trimethylammonium bromide (CTAB) protocol modified from Doyle and Doyle (1987). The barcode region of the COI (Mitochondrial cytochrome oxidase I) gene (COI-5P) was amplified with a single primer combination, GAZF1/GAZR1 (Saunders 2009). PCR reactions were prepared using Mango TaqTM DNA polymerase kit (Bioline, UK) according to manufacturer's instructions. Amplification conditions were set according Saunders and Moore (2013) and were carried out in a thermal cycler Mastercycler epgradient (Eppendorf, USA). PCR products from agarose gel were purified using the QIAquick® Gel Extraction Kit (Qiagen).

Alignment and phylogenetic analysis. Newly generated COI sequences were aligned with other GenBank sequences relevant to this study (Table 1) using CLUSTALW in MEGA 6 (Kumar et al. 2013) under default conditions. A Neighbor Joining (NJ) tree (10,000 replicates) of the COI barcode region and a pairwise distance matrix were obtained in MEGA 6 using the *p*-distance method. Final trees were edited using FigTree v. 1.4.2 and Adobe Illustrator (Adobe Systems Incorporated).

Results

New records. *Acanthophora dendroides*. USA, Florida, Fort Pierce, collected by T. Melton, A. Tronholm, M. DePriest & G. Garcia-Soto (The Phycolab, The University of Alabama), 13 August 2013, 1 individual, not vouchered (FK009, female). COI-5P barcode: GenBank accession MH388702. *A. dendroides* (as *A. aokii*). Philippine Islands, Dapdap, Tarangnan, collected by L. Liao, 22 April 1998, 1 individual, not vouchered

Table 1. Collection and voucher information/source and GenBank accession numbers of specimens included in this study. Sequences produced in this study are in bold

Species	Collection information	COI	Coll./Voucher
Acanthophora dendroides	USA, Florida, Fort Pierce, 10 Aug. 2013	MH388702	FK009
	Philippine Islands, Dapdap, Tarangnan, 22 Apr. 1998, L. Lawrence, (as *A. aokii*)	MH388739	UA0164
	Philippine Islands, Zamboanga City, 21 Apr. 1998, M. Hommersand, (as *A. aokii*)	MH388737	UA0162
	Australia, Western Australia, 17 Oct. 2009, R. Dixon	HQ959600	HUWAR022
A. pacifica	Sherwood et al. (2011)	HQ422729	ARS00619
	Sherwood et al. (2011)	HQ422947	ARS03733
A. spicifera	Venezuela, Falcon, Playa Buchuacos, 10 June 2012	MH388705	GG023
	Florida, Biscayne Bay, USA, 12 Aug. 2013, G. Garcia-Soto	MH388703	FK021
	Philippine Islands, Hilutungan, Cebu City, 19 Apr. 1998, M. Hommersand	MH388732	UA0150
	Philippine Islands, Maribago, Lapulapu City, Cebu prov., 16 Jan. 1993, L. Lawrence (as *A. aokii*)	MH388738	UA0163
	Sherwood et al. (2011).	HQ422873	ARS00126
	USA, Hawaii, Oahu, 11 Mar. 2008, Kurihara et al (2010)	GU223869	ARS03563
Acanthophora sp.	India, Mandapam, 2014, F. Bast	KJ934736	MDP-2014-AS-1
C. dangeardii	USA, Hawaii, Molokai, 21 Mar. 2008, K. Conklin	GU223879	
C. dasyphyla	USA, Florida, Fort Pierce, 10 Aug. 2013	MH388700	FK007
C. scintillans	France, Brittany, Le Loup, 30 Aug. 2011, L. Couceiro & M. Robuchon	KJ960537	
C. tenuissima	UK, England, Swanage, 7 Jun. 2015, P. Díaz	MF094021	PD2129
Chondria sp.	Brazil, Espírito Santo, Praia de Parati, 8 Apr. 2014, P. Díaz	MF093974	PD620
Coeloclonium sp.	Australia, South Australia, Port MacDonnell, 28 Aug. 1995, M. Hommersand	MH388746	UA0240

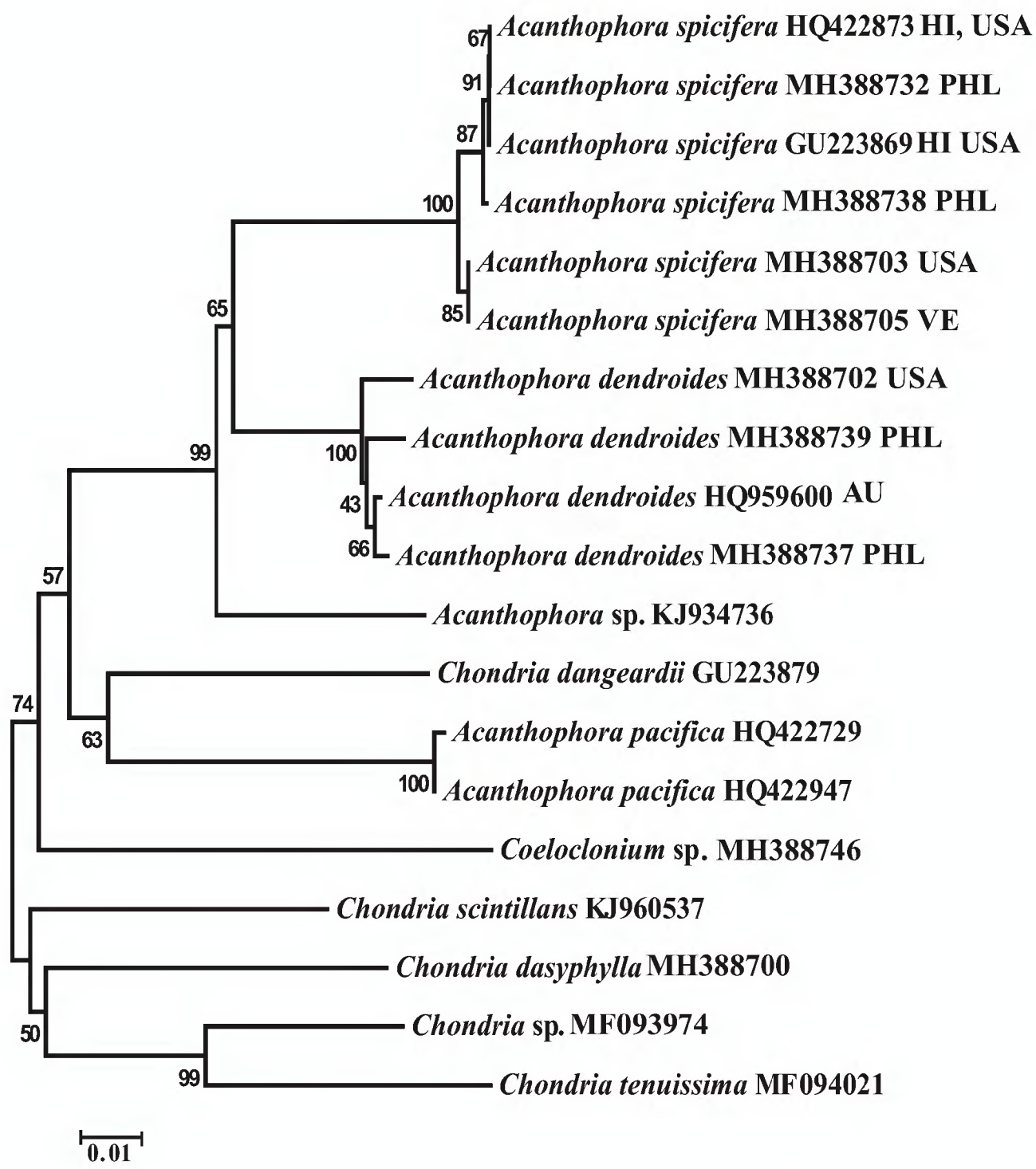

Figure 1. NJ tree based on *p*-distance method of the mitochondrion-encoded COI barcode region. Values at nodes represent bootstrap values (not shown if node received < 0.5).

(UA0162, tetrasporophyte). COI-5P barcode: GenBank accession MH388739; Zamboanga City, collected by M. Hommersand, 21 April 1998, 1 individual, not vouchered (UA0164, female). COI-5P barcode: GenBank accession MH388737.

Identification. Specimens were identified as *Acanthophora dendroides* in the BOLD identification engine where sequences clustered with a sample from Western Australia HUWAR022 (Fig. 1). Average intraspecific divergence of the COI-5P was 1%, with most of the variation attributed to specimens FK009 and UA0164. A morphological comparison with the major diagnostic features to delimit *A. dendroides* from other species is summarized in Table 2. These features include primordium type and the morphology of the apical zone according to Perrone et al. (2006). To a lesser extent,

Table 2. Comparison of vegetative characters among species of *Acanthophora** sensu De Jong et al. (1999) and Perrone et al. (2006).

	A. dendroides	***A. ramulosa***	***A. aokii***	***A. spicifera***	***A. nayadifomis***
Type of habit	Bushy	Slender	Bushy/fastigiate	Slender	Slender
Growth pattern	Monopodial	Monopodial	Monopodial	Monopodial to ramisympodial	Monopodial to ramisympodial
Type of apical region in main axes	Rod-like	Rod-like	Asparagus-like	Spadix-like	Rosette-like
Primordium type (high order laterals)	Rounded, *Coelochondria*-like, very large	Rounded, *Coelochondria*-like	Rounded, very small	Conical	Conical
Spines at terminal primordium	Large, closely arranged	Few, small, spaced	Spineless	Several spines	Several spines
Arrangement of primordia on main axes	Less spaced	Widely spaced	Widely spaced	Crowded	Crowded?
Lateral branch base	Constricted	Slightly constricted	Non-Constricted	Slightly Constricted	Non-constricted

**Acanthophora muscoides* is not included in the table and it is considered a taxon inquirendum sensu Perrone et al. 2006 (neotype and other herbarium specimens seem to belong to different species).

other features can be useful to discriminate between some of the species, such as spine development, tetrasporangial branches, and other overall habit anatomy features.

Acanthophora dendroides is characterized by having a bushy habit that is densely branched (Fig. 2A). Main axes and laterals in *A. dendroides* produce large spines, basally broad and regularly spaced throughout

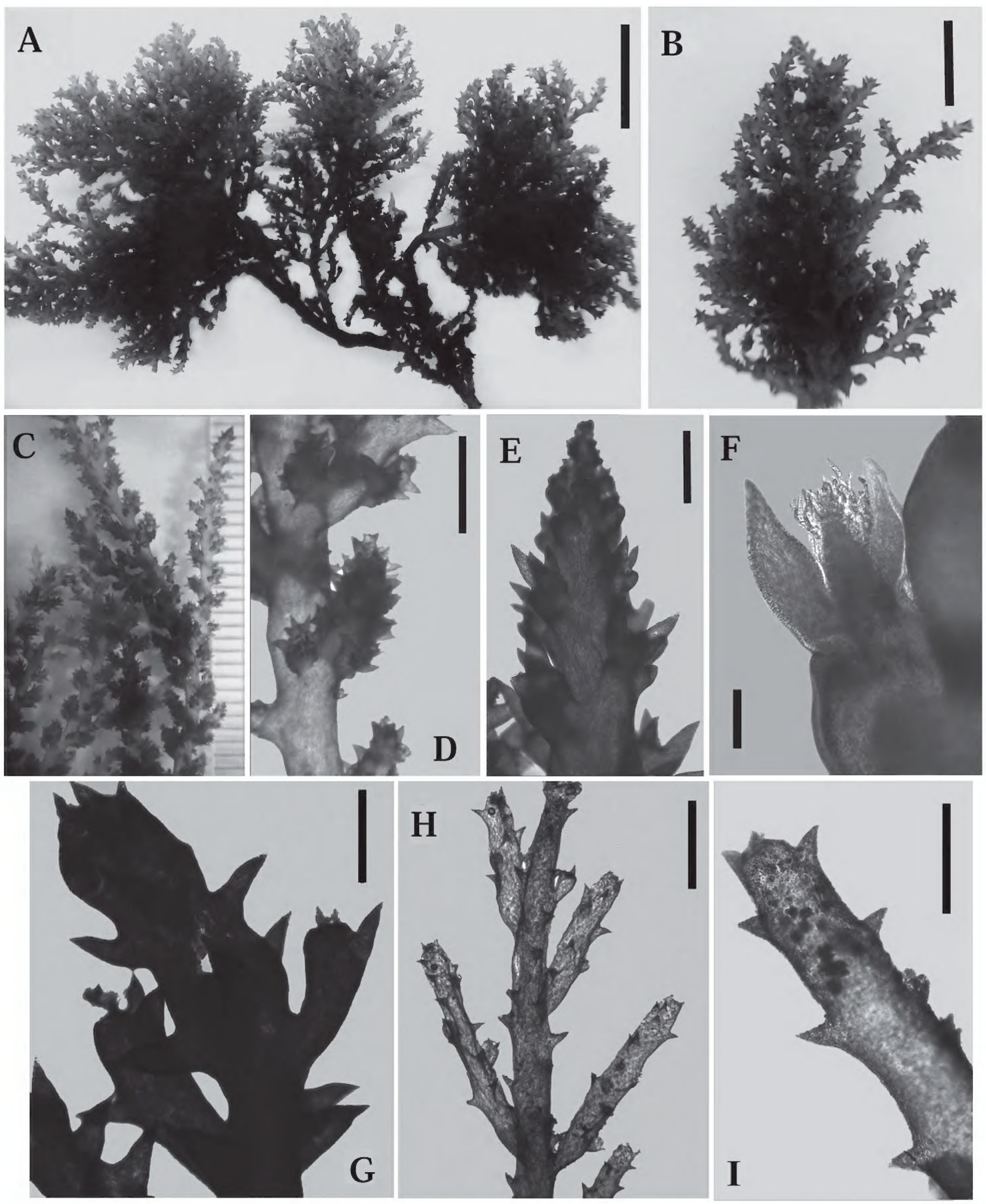

Figure 2. A, B. *Acanthophora dendroides* FK009. **A.** Habit detail of female specimen. Scale bar = 10 mm. **B.** Branch detail showing crowded arrangement of laterals. Scale bar = 5 mm. **C–F.** *A. spicifera* from Venezuela G023. **C.** Detail of habit of tetrasporophytic specimen showing indeterminate laterals and short ramuli with crowded arrangement of spines. Scale bar = 10 mm. **D.** Detail of a main lateral with no spines. Scale bar= 2 mm. **E.** Spadix-like apical region. Scale bar = 750 µM. **F.** Conical primordium with trichoblasts. Scale bar = 200 µM. **G–I.** *Acanthophora dendroides*. **G.** Detail of specimen FK009 main lateral showing apical zone with large terminal primordium and laterals with basal constriction. Scale bar = 500 µM. **H.** Detail of main lateral of specimen UA0162 showing basal constriction and regularly distributed spines. Scale bar = 2mm. **I.** Spinous tetrasporangial branch of UA0162. Scale bar = 500 µM.

the thallus; the main axes show few spiny outgrowths but spines are distributed in a more crowed fashion at distal parts (Fig. 2A, B). In contrast, the seemingly cosmopolitan species *A. spicifera* is distinguished by having usually spineless main axes (when sympodial growth occurs) clothed with spinous short laterals and indeterminate laterals producing short ramuli in the same fashion (Fig. 2C, D). However, these features are ultimately dependent on the type of growth (Perrone et al. 2006) and it is the spadix-like apical zone (Fig. 2E) and the production of a conical shape primordium (Fig. 2F) that distinguishes *A. spicifera* from *A. dendroides* (Table 2). *Acanthophora dendroides* and *A. ramulosa* Lindenberg ex Kützing from Angola are very similar and morphologically delimited by the degree of development of some vegetative features so that in the bushier *A. dendroides* the spines are basally broader and larger, the basal constriction of branches is more pronounced and the terminal primordia are larger (Fig. 2G). Thus, these 2 species do not appear to differ in any important respect but size. It is important to highlight that Womersley (2003) found that Australian specimens of *A. dendroides* (including Western Australia and an isotype specimens) did not show constricted laterals and they were scarcely "determinate". Furthermore, specimen UA0162 showed smaller spines when compared to UA0164 and FK009 (Fig. 2H, I). It was also evident that first order laterals and spines in specimen UA0164 are more spaced than in the bushy specimen FK009. However, this can be attributed to aging and the poor preservation of the samples.

Discussion

In this study, *Acanthophora dendroides* Harvey is reported for the first time in the Atlantic Ocean. Originally described from Western Australia (Rottnest Island), this species was geographically restricted to the Indian Ocean with records in Pakistan, Sri Lanka, India, Kenya, Tanzania, and Australia (Silva et al. 1987). *Acanthophora aokii*, *A. muscoides*, and *A. spicifera* have been recorded from several localities in the Philippines. However, the specimens of *A. aokii* included from the Philippines in this study are not morphologically congruent with *A. aokii* as originally described from the type locality at Tainan, Taiwan and for the Philippines (Cordero Jr 1977). *Acanthophora aokii* is a distinctive species that can be discriminated from *A. dendroides* by its small size (2–3 cm), the apical region morphology, fastigiated habit and minute primordia (Table 2). Consequently, the geographic range of *A. dendroides* is extended to the Philippines, accounting for the species' first occurrence in the Western Pacific Ocean. Morphological comparison between specimens from Philippines and Florida casts doubt about whether *A. ramulosa* and *A. dendroides* are distinct species. However, material of *A. ramulosa* from the type locality, Angola (Central Africa), is needed to ascertain its phylogenetic position. Likewise, the validation of morphological characters for species delimitation in the genus, such as apical zone morphology and other vegetative traits, awaits the inclusion of more species in future molecular analyses

It is worth noting that with the addition of new COI-5P sequences of *A. spicifera* (the generitype) from the Western Atlantic, *A. pacifica,* the only flattened species of the genus, clustered with a pacific sequence of *Chondria dangeardii* E.Y. Dawson, suggesting that the genus *Acanthophora* should be emended to comprise only cylindrical species. Previous molecular studies have also shown that *A. pacifica* is not phylogenetically related to *A. spicifera* from Hawaii (Kurihara et al. 2010, Sherwood et al. 2011, Diaz-Tapia et al. 2017). *A. pacifica* was transferred from *Cladhymenia pacifica* Setchell by Kraft (1979) and uncertainty about its taxonomic placement has been pointed out by several authors (Gordon-Mills and Womersley 1987, de Jong 1999, Perrone et al. 2006, N'Yeurt and Payri 2010).

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Authors' Contributions

GCGS participated in the collection of specimens, performed morphological and molecular analyses, interpreted the data and drafted the manuscript. JMLB interpreted the data and contributed to the draft and critical review of the final manuscript. Both authors read and approved the final manuscript.

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